

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: *Li et al.*

Group Art Unit: 1635

Serial No.: 10/574,129

Examiner: Bowman, Amy Hudson

Filed: November 2, 2006

Docket No.: 180/179 PCT/US

Confirmation No.: 9249

For: A NOVEL siRNA-BASED APPROACH TO TARGET THE HIF- α FACTOR FOR
GENE THERAPY

DECLARATION OF CHUAN LI, PH.D. PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. My name is Chuan Li, Ph.D., and I am Director of Molecular Radiation Oncology at the University of Colorado at Denver's School of Medicine.
2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit B**.
3. I am a co-inventor of the above captioned U.S. Patent Application Serial No. 10/574,129.
4. I have had an opportunity to review pending claims 36, 38-42, 45-59, 62, and 63 in the above captioned U.S. Patent Application Serial No. 10/574,129.

5. I have also had the opportunity to review the Non-Final Official Action dated December 18, 2008 (hereinafter the "Non-Final Official Action") from the United States Patent and Trademark Office (hereinafter "the Patent Office").

6. Various siRNAs were tested for their abilities to downregulate HIF-1 α activity by producing retroviruses that encode various siRNAs that target the human HIF-1 α mRNA sequence. Retroviruses encoding siRNA sequences targeted to the various sequences in the human HIF-1 α gene were produced that targeted the following human HIF-1 α nucleotide sequences:

ATGACATGAAAGCACAGAT (siRNA 1);

AACTGGACACAGTGTGTTT (siRNA 2);

AAATGAGAGAAATGCTTAC (siRNA 3); and

AAATGGCCTTGTAAG (siRNA 4).

7. siRNA 1 included a sense sequence that corresponded to SEQ ID NO: 7 of the above captioned U.S. Patent Application Serial No. 10/574,129. The other three siRNAs were targeted to sequences in the vicinity of the sequence targeted by siRNA 1 as shown in Exhibit C submitted herewith.

8. The retroviruses were used to infect the human colon cancer cell line HCT116. After infection, the cells were treated with puromycin, which selected for stable integration of the retrovirus genomes. After 5 day of selection, puromycin was washed off the cells, and the cells were subjected to hypoxia (0.5% O₂) treatment for 24 hours. After 24 hours treatment, the cells were lysed and assayed for HIF-1 activity using a commercially available kit purchased from Active Motif of Carlsbad, California.

9. The results of these assays are presented in Exhibit D submitted herewith. Normoxic cells containing each of the siRNAs were used as controls for normalization. Control indicated vector infected control cells.

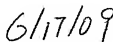
10. As can be seen in Exhibit D, the retrovirus that encoded an siRNA that included SEQ ID NO: 7 (*i.e.*, siRNA 1) showed superior ability to downregulate HIF-1 activity as compared to any of siRNAs 2-4. Specifically, cells containing siRNAs 2, 3, and 4 showed about 50-300% higher HIF-1 activity levels than cells containing siRNA 1.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,



Chuan Li, Ph.D.



Date

Attachments: Exhibits B-D

Exhibit B

Curriculum Vitae

Chuan-Yuan Li

Personal Information

Tel: (303) 724-1542

Fax: (303) 724-1554

Email: Chuan.Li@ucdenver.edu

Citizenship:

U.S. Citizen.

Education

B.S. in Chemistry, 1987, University of Science & Technology, Hefei, China.

Ph.D. in Cancer Biology, 1993, Harvard University, Cambridge, MA.

Professional Experience

- 2006- Professor of Pharmacology, University of Colorado Health Sciences Center, Aurora, CO.
- 2006- Professor and Director, Division of Radiation and Cancer Biology, Dept. of Radiation Oncology, University of Colorado Health Sciences Center, Denver, CO.
- 2005-2006 Professor, Departments of Radiation Oncology, Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC
- 2002-2005 Associate Professor, Dept. of Radiation Oncology, Duke University Medical Center, Durham, NC
- 1997-2002 Assistant Professor, Dept. of Radiation Oncology, Duke University Medical Center, Durham, NC
- 1993-1996 Postdoctoral research fellow, Harvard University, Boston, MA

Honors and Awards

- 1987-89 Rohm and Haas Fellowship for graduate study at Harvard University
- 1990 Student Travel Award, 39th Radiation Research Society Annual Meeting, New Orleans, LA
- 1991 Student Travel Award, 9th International Congress of Radiation Research, Toronto, CA
- 1993 Kresge Center for Environmental Science pilot grant, Harvard University
- 1999 Komen Foundation Grant for Breast Cancer Research
- 2001 Best Poster Award, Annual Meeting of the Duke University Cancer Center
- 2002 Best Proffered Paper, 6th Wolfsberg Symposium in Radiation Oncology, Ematingen, Switzerland, 2002
- 2006 Michael Fry Research Award from the Radiation Research Society, Philadelphia, PA.

Awards obtained by graduate students and postdoctoral fellows.

- 2005 AACR Travel Award, Fang Li, Postdoc Anaheim, CA
- 2005 RRS travel Award, Postdoc Fang Li
- 2005 RRS travel award, Postdoc Shanling Liu
- 2005 Excellence and Soundness of Research Methods award from the Fifth International Symposium on Therapeutic Ultrasound, Student: Yunbo Liu

Exhibit B

Curriculum Vitae

- 2006 Scholarship to attend the Keystone symposium in Hypoxia research for Fang Li, postdoc.
2006 Scholarship to attend AACR special conference on genetic susceptibility to cancer, Bin Yan.
2008 Travel Award for postdoc Wenrong Li to attend 54th annual meeting of RRS.

Membership in Professional Societies

Radiation Research Society, Member of the Educational Committee
American Association for Cancer Research
American Society of Gene Therapy
International Society of Stem Cell Research

Reviewership

Reviewers for Academic Journals:

Molecular Cell, Cancer Research, Clinic Cancer Research, Gene Therapy, J. of National Cancer Institute, Molecular Therapy, International J. of Cancer, J. of Investigative Dermatology, Int. J. of Hyperthermia, Int. J. of Radiation Oncology, Biology and Physics, Leukemia, etc.

Grant Review Committee Membership:

Ad hoc member, NIH study section of Metabolic Pathology, 2001-2003. Radiation Therapeutics and Biology (RTB), 2003-2004, 2007-present;
Komen Foundation for Breast Cancer Research, 2000
NASA Fundamental Space Biology Research Program, 2004, 2005
DOE Low Dose Radiation Research Program, 2004
Clinical and Basic Science Grant Review Panel, National Science Foundation of China, 2005-Present.

Invited Presentation(selected)

1. Elevated frequency of microsatellite mutations in human lymphocyte cells selected for mutation at the thymidine kinase locus, 41st Annual Meeting of the Radiation Research Society, Dallas, Texas, 1993.
2. Involvement of tumor suppressor genes in human cell transformation, Workshop on Neoplastic transformation in human cell systems in culture: Mechanisms of Carcinogenesis, Chicago, Illinois, 1995.
3. A Heat-Induced Gene Therapy Approach for Cancer Treatment, 46th Annual Meeting of the Radiation Research Society, Dallas, Texas, 1998.
4. Hyperthermia-regulated gene therapy, Annual meeting of the North America Hyperthermia Society, Philadelphia, PA, 1999
5. Combined radiation and gene therapy treatment of breast cancer in murine tumor models, Annual Research Meeting of the Komen Foundation for Breast Cancer Research, Washington DC, 2000
6. Progress in hyperthermia-regulated gene therapy (Keynote Address), The 17th Annual Meeting of the Japanese Society of Hyperthermic Oncology, Niigata, Japan, 2000
7. Heat-controlled gene therapy, International Conference of Stress Proteins in Biology and Medicine, Woods Hole, MA, 2000
8. Persistent genetic instability in cancer cells induced by non-DNA damaging stress, Gordon Conference on Radiation Oncology, Ventura City, CA, 2001

Exhibit B

Curriculum Vitae

9. Heat-induced transcriptional Targeting of therapeutic genes to cancer. 48th annual Meeting of the Radiation Research Society, Puerto Rico, 2001
10. Increased resistance of cancer cells mediated by the ILK gene. 49th annual meeting of the Radiation Research Society, Reno, NE, 2002
11. Tumor therapy by use of adenovirus vectors that target tumor cells genetically. 6th Wolfsberg Symposium in Radiation Oncology, Ematingen, Switzerland, 2002.
12. Telomerase-targeted oncolytic virus gene therapy. Annual Meeting of the North American Hyperthermia Society, Quebec City, Quebec, Canada. 2003.
13. Targeted gene therapy for cancer. 10th SCBA International Symposium, Beijing, China. 2004.
14. Minisymposium presentation, AACR annual meeting, Anaheim, CA, 2005.
15. Invited presentation, NIH conference on Bioimaging, DC, 2005
16. Invited presentation, Radiation Research Society, Denver, Colorado, 2005.
17. Invited Speaker, Division of Pharmaceutical Sciences, University of Wisconsin, Madison, 2006
18. Invited Speaker, Ontario Cancer Institute, Toronto, Canada, 2006

Teaching Activities

Cancer Biology (1997-1999), offered by DUMC Cancer Center, Primary Instructor: Dr. EC Halperin.

Radiation Biology(1997-2006), offered by Dept. of Radiation Oncology, DUMC; Primary Instructor: Chuan-Yuan Li.

Biomedical Engineering 260 (1997-2005), offered by Dept. of Biomedical Engineering, Duke University School of Engineering. Primary Instructor: Dr. F. Yuan.

Biology of Radiation Therapy, offered by Dept or Radiation Oncology, University of Colorado Denver School of Medicine. Primary Instructor: Chuan-Yuan Li.

Grant Support

Past Support

Development of a novel gene therapy approach based on hyperthermia. Duke Breast SPORE pilot project. Amount: \$18,000; Duration: 07/97-06/98. PI; Chuan-Yuan Li

Development of Biological Sensor for Stress exposure. Pilot project funded by the Duke University Marine Biology Center. Amount \$15,000. Duration 07/98-06/99. PI: Chuan-Yuan Li

Hyperthermia-controlled gene therapy. Celsion Cooperation. Amount: \$50,000; Duration 1998-1999. PI: Chuan-Yuan Li

A hyperthermia-mediated gene therapy approach for cancer. NIH 1R01CA81512. Amount: \$120,000/yr direct cost. Duration: 1998-2003. PI: Chuan-Yuan Li

Isolation and Characterization of Angiogenesis-related genes in the tumor vasculature. Glaxo-Wellcome-Duke Collaborative Research Agreement. Amount: \$300,000. 1999-2001. PI: Chuan-Yuan Li

Enhancement of breast cancer radiotherapy by genetic immunotherapy. Komen Foundation for Breast Cancer Research. Amount: \$250,000. Duration: 1999-2001. PI; Chuan-Yuan Li

Exhibit B

Curriculum Vitae

A Replication-competent Adenovirus That Targets Hypoxic Tumor Cells. Duke Comprehensive Cancer Center. Discovery Research Group Grant. Amount: \$30,000. Duration: 2002. PI: Chuan-Yuan Li.

A novel transgenic mouse model for studying tumor hypoxia and angiogenesis. Morris Cancer Foundation & NIH imaging Pilot grant. Amount: \$100,000. Duration: 2002-03. PI: Chuan-Yuan Li.

Hyperthermia and perfusion effects in cancer therapy. NIH 5P01 CA42745. Amount \$17,000,000. Duration: 2000-2005. PI: M.W. Dewhirst. 10% effort for C-Y. Li

SPORE in Breast Cancer. NIH 5P50-CA-68438. Amount: \$1,851,120. Duration: 2002-2007. PI: Lysterly. 15% Effort for C-Y. Li

Enhancement of Prostate Cancer Radotherapy by immunogene therapy. Department of Defense. Amount: \$215,000. Duration: 2002-2006. PI: Chuan-Yuan Li

Mechanistic studies of tumor therapy by electrofusion. NIH. Amount: 200K/yr annual direct cost. Duration: 2002-2007. PI: F. Yuan. 10% effort for C-Y. Li.

A novel adenovirus vector that targets telomerase-expressing breast cancer cells. Komen Foundation for Breast Cancer Research. Amount: \$250,000/3yrs. Duration: 2003-2007. PI: Chuan-Yuan Li

Molecular Dissection of the roles of SOD genes in mammalian cellular response to low dose radiation. DOE. Amount: \$900K for 3 years. 2003-2007. PI: C-Y. Li

Ultrasound-mediated gene delivery and activation. NIH. Amount \$1,250,000. Duration: 2003-2008. PI: Pei Zhong. 10% effort for C-Y. Li.

A hyperthermia-mediated gene therapy approach for cancer. NIH 1R01CA81512. Amount: \$150,000/yr direct cost. Duration: 2003-2009. PI: Chuan-Yuan Li

HZE particle induced persistent genetic instability/oncogenic transformation and their prevention. National Aeronautics and Space Administration (NASA). Amount: 200K/yrs. Duration: 2003-2008. PI: C-Y. Li

Imaging tumor hypoxia in a transgenic mouse model. NIH R21 EB001882. Amount: \$500,000 total. Duration: 2003-2007. PI: Chuan-Yuan Li

Active/Current Support

University of Colorado SPORE in Lung Cancer (NCI). PI: Paul Bunn. Amount: \$2,500,000/yr. Duration: 2008-2013. Part for C-Y. Li, Developmental Project: \$50,000/yr direct cost.

Exhibit B

Curriculum Vitae

Roles of HIF-1 genes in head and neck cancer radiotherapy (NIH/NCI). PI: Chuan-Yuan Li. Duration: 01/01/2009-12/31/2013. Amount: \$200K/yr direct, \$300K/yr total.

Mechanistic studies of space radiation induced carcinogenesis (NASA). PI: Chuan-Yuan Li, Duration: 06/01/2009-05/31/2010. Amount: \$220K/yr direct, \$354K/year total.

Mechanistic studies of tumor response to cytotoxic chemotherapy (NIH/NCI). Duration: 07/01/2009-06/30/2014; PI: Chuan-Yuan Li; Amount: 200K/yr direct, \$300K/yr total.

Publications

Peer-Reviewed Publications:

1. Li, C-Y., Yandell, D.W., and Little, J.B. Molecular Mechanisms of Spontaneous and Induced Loss of Heterozygosity in Human Cells In Vitro (1992). Somatic Cell and Molecular Genetics, Vol. 18, 77-87.
2. Li, C-Y., Yandell, D.W., and Little, J.B. Evidence for Coincident Mutations in Human Lymphoblast Clones Selected for Functional Loss of a Thymidine Kinase Gene(1992). Molecular Carcinogenesis, Vol. 5, 270-277.
3. Spiro, I. J., Yandell, D.W., Li, C-Y. , Saini, S., Ferry, J., Powelson J., Katkov, W.N., and Cosimi, A.B. Lymphoma of Donor Origin Occuring at the Porta Hepatis of Transplanted Liver(1993). New England Journal of Medicine, Vol.329, 27-29.
4. Poremba, C., Dockhorn-Dworniczak, B., Merrit, V., Li, C-Y., Heidle, G., Tauber, P., Bocker, W., , and Yandell, D.W. Immature Teratomas of Different Origin Carried by a Pregnant Mother and Her Fetus(1993). Diagnostic Molecular Pathology , Vol.2, 131-136.
5. Li, C-Y., Yandell, D.W., and Little, J.B. Elevated frequency of Microsatellite Mutations in Human Lymphoblast Clones Selected for Mutations at Thymidine Kinase locus(1994). Molecular & Cellular Biology, Vol. 14, 4373-4379.
6. Li, C-Y., Nagasawa, H., and Little, J.B. Confluent Holding Recovery in Irradiated Human Diploid Fibroblasts: Possible Role of the p53/Waf1 Signal Transduction Pathway(1995). Journal of Cellular Biochemistry, (S) 21A, 340.
7. Li, C-Y., Nagasawa, H., Tsang, N., and Little, J.B. Radiation-Induced Irreversible G0/G1 Block is Abolished in Human Diploid Fibroblasts Transfected with the Human Papilloma Virus E6 gene: Implication of the p53-Cip1/WAF1 pathway (1995). International Journal of Oncology, Vol. 223-236.
8. Li, C-Y., Suardet, L., and Little, J.B. Potential Role of WAF1/Cip1/p21 as a Mediator of TGF- β Cytoinhibitory Effect . Journal of Biological Chemistry, Vol. 270 No. 10., 1995 pp4971-4974.

Exhibit B

Curriculum Vitae

9. Nagasawa, H., Li, C-Y., Maki, C.G. , Imrich A. and Little, J.B. Relationship between Radiation-Induced G1 Phase Arrest and p53 Function in Human Tumor Cells *Cancer Research* vol 55 No. 9, 1995, pp1842-1846.
10. Little, J.B. , Nagasawa, H., Keng, P., and Li., C-Y. Absence of Radiation-induced G1-arrest in Two Closely Related Human Lymphoblast Cell Lines That Differ in p53 Status *Journal of Biological Chemistry* Vol.270, No. 19, 1995 pp11033-11036.
11. Tsang, N., Nagasawa, H., Li, C-Y., and Little, J.B. Abrogation of p53 function by Transfection of HPV16 E6 Gene Enhances the Resistance of Human Diploid Fibroblasts to Ionizing Radiation *Oncogene* vol. 10, 1995, pp2403-2408.
12. Li, C-Y., Nagasawa, H., Dahlberg, W.A., and Little, J.B. Diminished Capacity for p53 in Mediating a Radiation-Induced G1 Arrest in Established Human Tumor Cell Lines *Oncogene* vol. 9, No. 9, 1995, pp1885-1892.
13. Little, J.B., Li, C-Y., Nagasawa, H., Pfenning, T., and Vetrovs, H. Genomic Instability and Radiation Mutagenesis(1996) *J. Chimie Phys.* vol. 93, 157-164.
14. Li, C-Y, Nagasawa, H., Dahlberg, W.A., and Little, J.B. The Role of Tumor Suppressor Genes in Determining a Radiation-induced G1-arrest and Human Cell Carcinogenesis(1996) *Radiation Oncology Investigations*, vol. 3, 268-271.
15. Huang, H-M., Li, C-Y, Little, J.B. Abrogation of p53 Function in Human Tumor Cells Does Not Alter Their Sensitivity to Ionizing Radiation(1996). *International Journal of Radiation Biology*, vol. 70 (2), 151-160.
16. Suardet, L., Li, C-Y., and Little J.B. Radio-induced modulation of transforming growth factor sensitivity in a p53 wild-type human colorectal cancer cell line(1996). *International Journal of Cancer* vol. 68(1), 126-31.
17. Huang, Q., Tao., Y., Li, C-Y., and Yandell, D. The status of p16 and p15 in primary tumors and cell lines (1996). *Chinese Journal of Medical Genetics*, vol. 13, 198-202.
18. Yu, Y., Li, C-Y., Little, J.B. Abrogation of p53 function by HPV16 E6 gene delays apoptosis and enhances mutagenesis but does not alter radiosensitivity in TK6 human lymphoblastoid cells(1997). *Oncogene*, vol. 14(14), 1661-1667.
19. Little JB, Li C-Y, Nagasawa H, Huang H. Influence of p53 expression on radiosensitivity of human normal and tumor cells(1998). *J CHIM PHYS* vol 95,820-829.
20. Tao, Y., Huang, Q., Li, C-Y., and Yandell, D. Deletions and point mutations of p16, p15 gene in primary tumors and tumor cell lines. (1999). *Chinese Med Sci. J.* vol.14, 200-235.
21. Huang, Q., Shan, S, Braun, R.D., Lanzen, J., Anyrhambatla, G., Kong, G., Borelli, M., Corry, P., Dewhirst, M.W., and Li, C-Y. GFP based non-invasive, in vivo monitoring of gene expression(1999). *Nature Biotechnology*, vol 17, 1033-1035.

Exhibit B

Curriculum Vitae

22. Li, C-Y., Huang, Q., Braun, R.D., Lanzen, J., Hu, K., Lin, P., Dewhirst, M.W. Initial stages of tumor cells-induced angiogenesis: evaluation via skin window chambers in rodent models(2000) *Journal of the National Cancer Institute*, vol 92, 143-127.
23. Huang, Q., Li, C-Y. Comparative studies of GFP gene transgene by viral and non-viral approaches (2002). *Chinese J. Pathology*. Vol 29, 143-144.
24. Huang, Q., Li, C-Y. Dynamic observation of tumor cell growth by use of GFP-transduced tumor cells (2000). *Chinese J. Pathology*. Vol 29, 230-232.
25. Huang, Q., Xu, P., Liu, WW., Wang, F., Gu, Q., Tian, SH., Fan, Y., Xie, KC., Chen, XF., Li, C-Y. Preparation of green fluorescent protein retrovirus and its application in mediating gene transfer into retinal pigment epithelial cells (2001). *Chinese J. of Ophthalmology*, vol. 37, 248-251.
26. Li, C-Y., Huang, Q., Braun, R.D., Lanzen, J., Hu, K., Lin, P., Dewhirst, M.W. Re: Initial stages of tumor cell-induced angiogenesis: Evaluation via skin window chambers in rodent models - Response(2000). *Journal of the National Cancer Institute*, Vol. 92, 1445-1446.
27. Huang, Q., Hu, K., Lohr, F., Zhang, L., Braun, R., Lanzen, J., Little, J.B., Dewhirst, M.W., and Li, C-Y. Heat-induced Gene Expression as a Novel Targeted Cancer Gene Therapy Strategy.(2000) *Cancer Research*, vol. 60, 3435-3439.
28. Lohr, F., Hu, K., Huang, Q., Zhang, Li., Dewhirst, M.W., and Li, C-Y. Enhancement of Radiotherapy by Hyperthermia-regulated Gene Therapy.(2000). *International Journal of Radiation Oncology, Biology and Physics*. Vol.48, 1513-1518.
29. Lohr, F., Hu, K., Haroon, Z., Samulski, TV., Huang, Q., Beaty, J., Dewhirst, M.W., and Li, C-Y. Combination treatment of murine tumors by adenovirus mediated local B7/IL12 immunotherapy and radiotherapy(2000). *Molecular Therapy*, vol2, 195-203.
30. Li, C-Y., Shan, S., Cao, Y., Dewhirst, M.W. Role of incipient angiogenesis in cancer metastasis.(2000). *Cancer Metastasis Reviews*, vol 19, 7-11.
31. Li, C-Y., Little, J.B. Hu, K., Zhang, W., Zhang, L., Dewhirst, M.W., Huang, Q. Persistent genetic instability in cancer cells induced by non-DNA damaging stress exposures (2001). *Cancer Research* vol 60, 428-432
32. Zhang, X., and Li, C-Y. Generation of recombinant adeno-associated virus vectors by a complete adenovirus-mediated approach(2001). *Molecular Therapy* vol.3, 787-792.
33. Xie, KC., Xu, P., Gu, Q., Liu, WW., Wang, F., Tian SH., Chen, XF., Li, C-Y., Huang, Q. Using GFP retrovirus to label tumor cells and vascular endothelial cells. *Chinese J. Lung Cancer*. Vol 4, 20-24.
34. Lohr, F., Lo, D., Zaharoff, DA., Hu, K., Li, Y., Zhang, X., Dewhirst, M.W., Yuan, F., and Li, C-Y. Effective tumor therapy with plasmid-encoded IL12 and IL2 combined with in vivo electroporation (2001). *Cancer Research* vol 61, 3282-3285

Exhibit B

Curriculum Vitae

35. Huang, Q., and Li, C-Y. Using hsp70 promoter to regulate target gene expression in tumor. *Chinese J. of Pathology*, vol. 30, 198-201.
36. Lohr, F., Huang, Q., Hu, K., Dewhirst, MW., and Li, C-Y. An inducible method for preventing systematic effects of toxic therapeutic genes delivered by intratumorally injected adenovirus vectors. *Clinical Cancer Research*. Vol. 11, 3625-3628
37. Zhang, X., Hu, K., and Li, C-Y. Protection against oxidized LDL-induced vascular endothelial cell death by integrin-linked kinase (2001). *Circulation* vol 104, 2762-2766.
38. Zaharoff, D., Barr, R., Li, C-Y., Yuan, F. Mechanistic studies of electric pulse mediated gene transfer in murine tumors (2002). *Gene Therapy*. Vol. 9, 1286-1290.
39. Wang, F., Chen, X., Tian, Y., Wu, J., Li, L., Li, C-Y., Huang, Q. Target gene transfer mediated by electroporation for cancer therapy in vivo (2002). *Progress in Biochemistry and Biophysics*. Vol. 29, 734-740.
40. Li, C-Y., and Dewhirst, M.W. Hyperthermia-regulated immunogene therapy (2002). *International Journal of Hyperthermia* Vol. 18, 589-96.
41. Dewhirst, M.W., Cao, Y., Moeller, B., Li, C-Y. Intravital Fluorescence facilitates the measurement of multiple physiological parameters and gene expression in tumors of living animals. *Disease Markers*. 18 (5-6): 293-311 2002
42. Azzam, EI, Nagasawa, N., Yu, Y., Li, C-Y. and Little, JB. Cell Cycle Deregulation and XPC Cell Transformation (2002). *Journal of Investigative Dermatology*. Vol 119, 1350-1354.
43. Baker-LePain, J.C., Sarzotti-Kelsoe, M., Fields, T.A., Li, C-Y., Nicchita, C.V. GRP94(gp96) and GRP94 N-Terminal binding domain elicit tissue non-restricted tumor suppression.(2002) *Journal of Experimental Medicine* Vol. 196, 1447-59.
44. Wang, F., Tian, Y., Li, L., Chen, X., Hu, HH. Li, C-Y., Huang, Q. Inhibition of tumor angiogenesis, growth, and metastasis by blocking VEGF paracrine pathway (2002). *Acta Biochimica et Biophysica Sinica*, vol 34., 165-170.
45. Zhang, X., Li, Y., Yan, B., Huang, Q., Dewhirst, M.W., Li, C-Y. Increased resistance of tumor cells to hyperthermia mediated by integrin-linked kinase (2003). *Clinical Cancer Research* Vol 9, 1155-60.
46. Wang, Y., Hu, JK., Krol, A., Li, Y-P., Li, C-Y., Yuan, F. Systemic dissemination of viral vectors during intratumoral injection.(2003). *Molecular Cancer Therapeutics*. Vol. 1233-1241.
47. Wang, F., Wu, J., Tian, Y., Chen, X., Hu, H., Wu, W., Li, C-Y., and Huang, Q. Role of VEGF in the growth and metastasis of a murine bladder carcinoma. (2003) *Chinese Science Bulletin*. Vol 48:2404-2410.

Exhibit B

Curriculum Vitae

48. Huang, Q., Wang, H., Zhang, X.W., Yan, B., Dewhirst, MW., and Li, C-Y. A conditionally replicative adenovirus targeted to telomerase-positive cancer cells. (2004)*Clinical Cancer Research*. Vol: 4:1439-1445
49. Shan, S., Robson, N. D., Cao, Y., Qiao, T., Li, C. Y., Kontos, C. D., Garcia-Blanco, M., Dewhirst, M. W. Responses of vascular endothelial cells to angiogenic signaling are important for tumor cell survival.(2004) *Faseb Journal*. Vol : 17(15),published online.
50. Zhang, X., Huang, Q., Li, Y., Yang, H., and Li, C-Y. GW112, a novel gene involved in the survival of cancer cells under stressful conditions. (2004)*Cancer Research*. Vol. 64: 2474-2481.
51. Moeller, B. J.; Cao, Y., Li, C. Y., Dewhirst, M. W. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors; Role of reoxygenation, free radicals, and stress granules (2004) *Cancer Cell*. Vol 5: 429-441.
52. Moeller, B.J., Cao, Y., Vujaskovic, Z., Li, C.Y., Haroon, Z.A., Dewhirst, M. W.(2004) The relationship between hypoxia and angiogenesis. *Seminars in Radiation Oncology*. Vol 14:215-21.
53. Herskind C, Fleckenstein K, Lohr J, Li CY, Wenz F, Lohr F (2004) Antitumoral action of interferons and interleukins in combination with radiotherapy. Part I: Immunologic basis. *STRAHLENTHERAPIE UND ONKOLOGIE* 180 (4): 187-193
54. Herskind C, Fleckenstein K, Lohr J, Li CY, Frederik W, Lohr F. (2004) Antitumoral action of Interferons and interleukins in combination with radiotherapy. part II: Radiobiological and immunologic strategies. *STRAHLENTHERAPIE UND ONKOLOGIE* 180 (6): 331-339
55. Zhang,X., Wang, H., Kon, T., Huang, Q., Dewhirst, MW., and Li, C-Y. Enhancement of tumor cell death in vitro and radiation therapy in vivo by use of an siRNA targeted to HIF1- α (2004). *Cancer Research*. Vol 64:8139-42.
56. V. Rao, P. Deng, R. Maddala, D. Epstein, C-Y. Li, Hiroaki Shimokawa (2005) Expression of dominant negative Rho-binding domain of Rho-kinase in organ cultured human eye anterior segments increases aqueous humor outflow. *Molecular Vision*, Vol 11: 288-97.
57. Wang, Y., Yang, Z., Liu, S., Krol, A., Li, C-Y., and Yuan, F (2005). Characterization of systemic adenovirus leakage during cancer gene therapy. *British Journal of Cancer*. Vol 92:1414-20.
58. Li, C. Y., Huang, Q., Kung, H. F. (2005) Cytokine and immuno-gene therapy for solid tumors. *Cell & Molecular Immunology*. Vol 2: 81-91.
59. Cao, Y., Li, C-Y., Moeller, B.J., Yu, D., Zhao' Y, Dreher,M., Shan., S., and Mark W. Dewhirst(2005). Observation of incipient angiogenesis that is independent of hypoxia and HIF-1 activation *Cancer Research*. Vol. 65: 5498-5505
60. Moeller, B., Dreher, M., Rabbani, Z., Schroeder, T., Cao, Y., Li, CY., and Dewhirst, M.W. Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity(2005). *Cancer Cell*. Vol. 8: 99-110.

Exhibit B

Curriculum Vitae

61. Wang, Y., Yang, Z., Liu, S., Krol, A., Li, C-Y., and Yuan, F (2005). A novel method for viral gene delivery in vivo.(2005) *Cancer Research* Vol 65: 7541-45.
62. Liu, S., Wang, H, Kon, T. and Li, C-Y. Significant enhancement of cancer radiation therapy by use of adenovirus-mediated secreted GRP94/gp96 expression. (2005) *Cancer Research* vol 65: 9126-9131
63. Plathow, C., Lohr, F., Divkovic, G., Rademaker, G., Farhan, N., Peschke, P., Zuna, I., Juergen D., Claussen, C., Kauczor, H., Li, C-Y., Jenne, J., Huber, P. Focal Gene Induction in the Liver of Rats by a Heat-Inducible Promoter Using Focused Ultrasound Hyperthermia: Preliminary Results.(2005) *Investigative Radiology* 40:729-735.
64. Liu, Y. Kon, T., Li, C-Y., Zhong, P. HIFU-gene activation. *Journal of the Acoustic Society of American* (2005) Vol 118: 3328-3336.
65. Mi, J., Sarraf-Yazdi, S., Zhang, X., Cao, Y., Dewhirst, M. W., Kontos, C. D., Li, C-Y. and Clary, B. M. A Comparison of Antiangiogenic Therapies for the Prevention of Liver Metastases (2006) *J of Surg Res*, 131:97-104.
66. Yan, B., Wang, H., Kon, T., and Li, C-Y. Pim-1 kinase inhibits the activation of reporter gene expression in Elk-1 and c-Fos reporting systems but not the endogenous gene expression: an artifact of the reporter gene assay by transient co-transfection. *Brazilian Journal of Medical and Biological Research* (2006) 39:169-176.
67. Yan, B. Wang, H., Peng, Y., Hu, Y., Wang, H., Zhang, X., Chen, Q., Bedford, J.S., Dewhirst, M.W., and Li, C-Y. Apoptotic DNA fragmentation as a checkpoint for genomic instability. *Proceedings of the National Academy of Sciences* vol(2006) 103:1504-1509.
68. Chuang, E.Y., Chen, X., Tsai, M., Yan, H., Li, C-Y., Mitchell, JB., Nagasawa, H., Wilson, PF., Peng, P., Bedford, JS., and Little, JB. Abnormal Gene Expression Profiles in Unaffected Parents of Patients with Hereditary Type Retinoblastoma. *Cancer Research* (2006) 66: 3428-3433.
69. Wang, Y., Wang, H., Li, C-Y., Yuan, F. Effects of rate, volume, and dose of intratumoral infusion on virus dissemination in local gene delivery. *Molecular Cancer Therapy* (2006) 5:362-366.
70. Yan, B., Wang, H., Zhuo, D., Li, F., Kon, T., Dewhirst, M.W., and Li, C-Y. Apoptotic DNA fragmentation factor maintains chromosome stability in a p53-dependent manner (2006). *Oncogene* 29(36):5370-5376.
71. Siddiqui F, Li CY, Zhang XW, Larue SM, Dewhirst MW, Ullrich RL, Avery PR Characterization of a recombinant adenovirus vector encoding heat-inducible feline interleukin-12 for use in hyperthermia-induced gene-therapy (2006). *International Journal of Hyperthermia* 22:117-134.

Exhibit B

Curriculum Vitae

72. Liu, Y. Kon, T., Li, C-Y., Zhong, P. High intensity focused ultrasound induced gene activation in solid tumors (2006), *Journal of the Acoustic Society of America* 120:492-501.
73. Yan, B., Wang, H., Li, F., Li, C-Y. Regulation of mammalian horizontal gene transfer by apoptotic DNA fragmentation (2006), *British Journal of Cancer*, 96:1696-1700.
74. Siddiqui F , Ehrhart EJ, Charles B, Chubb L, Li CY, Zhang XW , Larue SM, Avery PR, Dewhirst MW, Ullrich RL. Anti-angiogenic effects of interleukin-12 delivered by a novel hyperthermia induced gene construct (2006), *International Journal of Hyperthermia*, 22:587-606.
75. Yan, B., Wang, H., Rabbani, Z., Zhao, Y., Li, W., Yuan, Y., Li, F., Dewhirst, MW., and Li, C-Y. Tumor necrosis factor alpha is a potent endogenous mutagen that promotes cellular transformation (2006). *Cancer Research*, 66:11565-11570.
76. Siddiqui, F., Li, C-Y., LaRue, SM., Poulson, JM., Avery, P.R., Pruitt, A.F., Zhang, X., Ullrich, R.L., Thrall, D.E., Dewhirst, M.W., and Hauck, M.L. A phase I hyperthermia-induced interleukin-12 gene therapy trial in spontaneously arising soft tissue sarcomas (2007). *Molecular Cancer Therapeutics*, 6:380-389.
77. Cao Y., Sonveaux, P., Liu, S., Zhao, Y., Mi, J., Clary, B.M., Li, C-Y., Kontos, C.D., and Dewhirst, M.W. Systemic overexpression of angiopoietin-2 promotes tumor microvessel regression, inhibits angiogenesis and tumor growth (2007), *Cancer Research* 67:3835-3844.
78. Li, F., Sonveaux, P., Rabbani, P., Liu, S., Yan, B., Huang, Q., Vujaskovic, Z., Dewhirst, M.W., Li, C-Y. Regulation of HIF-1 α through S-nitrosylation (2007). *Molecular Cell*, 26:53-74.
79. Dewhirst, M. W., Cao, Y., Li, C. Y., Moeller, B. Exploring the role of HIF-1 in early angiogenesis and response to radiotherapy (2007). *Radiother Oncol* 83:249-255.
80. Li, W., Li, F., Huang, Q., Fredrick, B.A., Bao, S., and Li, C-Y. Non-invasive imaging and quantification of EGFR pathway activation in vivo. (2008). *Cancer Research*. 68:4990-97
81. Siddiqui, F., Avery, P. R., Li, C. Y., Zhang, X., LaRue, S. M., Dewhirst, M. W., Ullrich, R. L. Induction of the human heat shock promoter HSP70B by nutritional stress: implications for cancer gene therapy (2008). *Cancer Investigation* 26:553-61.
82. Wu, J., Zhang, S., Wu, X., Dong, X., Xu, P., Liu, X., Li, C-Y., Huang, Q. Enhanced transduction and improved photoreceptor survival of retinal degeneration by the combinatorial use of rAAV2 with a lower dose of adenovirus (2008). *Vision Research* 48:1648-1654.
83. Zhang, S., Wu, J., Wu, X., Dong, X., Liu, X., Li, C-Y., and Huang, Q., Distinctive gene transduction efficiencies of commonly used viral vectors in the retina (2008). *Current Eye Research* 33:81-90.
84. Vijayalakshmi Sridharan¹, Jason Guichard², Chuan-Yuan Li, Robin Muise-Helmricks, Craig Beeson³, and Gary L. Wright^{4*}. The oxygen-sensing signal cascade: clamping of O₂ respiration, reduced ATP utilization and inducible fumarate respiration (2008). *American Journal of Physiology (Cell Physiology)* 295:29-37

Exhibit B

Curriculum Vitae

85. Viola, R., Li, F., Li, C-Y., Provenzale, J., and Mark Dewhirst In vivo Bioluminescence Imaging Monitoring of Hypoxia inducible factor-1α, a hypoxia-inducible promoter that protects cells in Response to Chemotherapy (2008). *American Journal of Roentgenology* 191: 1779-1784.
86. He, Z., Li, F., Liu, B., Fan, Z., Huang, Q., Shen, J., Li, W., Bao, S., and Li, C-Y. Imaging caspase activities based on the proteasome system (2008). *Oncogene* (in review).
87. Kon, T., Huang, Q., Zhang X., Yang, Z., Kirkpatrick, J. and Li, C-Y. Targeted sensitization of cancer cells to radiation therapy by use of siRNA-based gene therapy. *International Journal of Radiation Oncology, Biology and Physics* (in review).

Book Chapters and other non-peer-reviewed articles

1. Little, J.B., Benjamin, M.B., Li, C-Y., and Yandell, D.W. Molecular Mechanisms of Radiation Induced Mutagenesis in Human Cells. In: C.B Seymour & C. Mothersill (ed), *New Developments in Fundamental & Applied Radiobiology*, Taylor & Francis, London, 1991, pp. 125-13
2. Li, C-Y. Progress in hyperthermia-regulated gene therapy (2000). *Japanese Journal of Hyperthermic Oncology* vol 16. Supplement. pp37-41.
3. Li, C-Y., Cao, YT, and Dewhirst, M.W. Applications of the green fluorescent protein (gfp) and its variants in tumor angiogenesis and physiology studies. In MA Mycek & B. Pogue (ed), *Fluorescence in Biomedicine*. Marcel Dekker, New York. (in Press)
4. Dewhirst, MW., Jone, E., Samulski, T., Vujaskovic, Z., Li, C., Prosnitz, L. Hyperthermia (2003) in D Kufe, R Pollock, R Weichselbaum, R Bast, T Gansler, J Hollan, E Frei III *Cancer Medicine*. BC Decker, Hamilton, London. (6th Edition). Pp623-636.
5. Lohr, F., Herskind, C., Lohr, J., Wenz, F., and Li, C-Y. Enhancement of the Radiation Response with Interleukins/Interferons (2004). In C. Nieder, L. Mikas, K. Ang *Modifiers of Radiation Response* (Springer, Berlin)
6. Yan, B., Peng, Y., and Li, C-Y. Molecular analysis of genomic instability caused by chronic inflammation (2008). In S. Kozlov *Methods in Molecular Medicine: Inflammation and Cancer*. (Humana Press, Totowa, NJ).

Exhibit C

SEQ ID NO: 1 - Human HIF-1 α Coding Sequence

```

atg gag ggc gcc ggc ggc gcg aac gac aag aaa aag ata agt tct gaa
Met Glu Gly Ala Gly Gly Ala Asn Asp Lys Lys Lys Ile Ser Ser Glu
                    5                                10                    15

cgt cga aaa gaa aag tct cga gat gca gcc aga tct cgg cga agt aaa
Arg Arg Lys Glu Lys Ser Arg Asp Ala Ala Arg Ser Arg Arg Ser Lys
                    20                                25                    30

gaa tct gaa gtt ttt tat gag ctt gct cat cag ttg cca ctt cca cat
Glu Ser Glu Val Phe Tyr Glu Leu Ala His Gln Leu Pro Leu Pro His
                    35                                40                    45

aat gtg agt tcg cat ctt gat aag gcc tct gtg atg agg ctt acc atc
Asn Val Ser Ser His Leu Asp Lys Ala Ser Val Met Arg Leu Thr Ile
                    50                                55                                60

agc tat ttg cgt gtg agg aaa ctt ctg gat gct ggt gat ttg gat att
Ser Tyr Leu Arg Val Arg Lys Leu Leu Asp Ala Gly Asp Leu Asp Ile
65                                70                                75                                80

```

SEQ ID NO: 7 (siRNA 1)

```

gaa gAT GAC ATG AAA GCA CAG ATg aat tgc ttt tat ttg aaa gcc ttg
Glu Asp Asp Met Lys Ala Gln Met Asn Cys Phe Tyr Leu Lys Ala Leu
                    85                                90                    95

gat ggt ttt gtt atg gtt ctc aca gat gat ggt gac atg att tac att
Asp Gly Phe Val Met Val Leu Thr Asp Asp Gly Asp Met Ile Tyr Ile
                    100                               105                               110

tct gat aat gtg aac aaa tac atg gga tta act cag ttt gaa cta act
Ser Asp Asn Val Asn Lys Tyr Met Gly Leu Thr Gln Phe Glu Leu Thr
                    115                               120                               125

```

siRNA 2

```

gga cac agt gtg ttt gat ttt act cat cca tgt gac cat gag gaa atg
Gly His Ser Val Phe Asp Phe Thr His Pro Cys Asp His Glu Glu Met
130                               135                               140

```

siRNA 3

```

aga gaa atg ctt aca cac aga aat ggc ctt gtg aaa aag ggt aaa gaa
Arg Glu Met Leu Thr His Arg Asn Gly Leu Val Lys Lys Gly Lys Glu
145                               150                               155                               160

```

siRNA 4

```

caa aac aca cag cga agc ttt ttt ctc aga atg aag tgt acc cta act
Gln Asn Thr Gln Arg Ser Phe Phe Leu Arg Met Lys Cys Thr Leu Thr
                    165                               170                               175

```


Exhibit D

